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► **To cite this version:**

Caterina Morcia, Mauro Malnati, Valeria Terzi. In vitro antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens.. Food Additives and Contaminants, 2012, pp.1. 10.1080/19440049.2011.643458 . hal-00778226

HAL Id: hal-00778226

<https://hal.science/hal-00778226>

Submitted on 19 Jan 2013

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In vitro antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens.

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-140.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	10-Oct-2011
Complete List of Authors:	Morcia, Caterina; CRA GPG Malnati, Mauro; Istituto Scientifico San Raffaele Terzi, Valeria; CRA GPG
Methods/Techniques:	Bioassay, Mycology
Additives/Contaminants:	Mycotoxins - trichothecenes, Mycotoxins
Food Types:	Cereals and grain
Abstract:	The aim of this study was to examine the effect of five natural molecules, taking part in the composition of essential oils, on ten different species of mycotoxigenic fungi involved in several plant diseases. The antifungal activities of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol were observed in vitro on <i>Fusarium subglutinans</i> , <i>Fusarium cerealis</i> , <i>Fusarium verticillioides</i> , <i>Fusarium proliferatum</i> , <i>Fusarium oxysporum</i> , <i>Fusarium sporotrichioides</i> , <i>Aspergillus tubingensis</i> , <i>Aspergillus carbonarius</i> , <i>Alternaria alternata</i> , <i>Penicillium</i> . The natural molecules tested showed toxic effects on in vitro mycelium growth of all the fungal strains. The five oil components tested have different level of potency. The results obtained are encouraging for further studies directed to the investigation of in planta antifungal activities of these essential oils components.

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5 ***In vitro* antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole**
6 **(eucalyptol) and thymol against mycotoxigenic plant pathogens.**
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34 **Abstract**
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37 The aim of this study was to examine the effect of five naturally occurring compounds from
38 essential oils, on ten different species of mycotoxigenic fungi involved in several plant
39 diseases. The antifungal activities of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol)
40 and thymol were observed *in vitro* on *Fusarium subglutinans*, *Fusarium cerealis*, *Fusarium*
41 *verticillioides*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Fusarium sporotrichioides*,
42 *Aspergillus tubingensis*, *Aspergillus carbonarius*, *Alternaria alternata*, *Penicillium*. The
43 naturally occurring compounds tested showed toxic effects on *in vitro* mycelium growth of all
44 the fungal strains. The five oil components tested had different level of potency. The results
45 obtained are encouraging for further studies directed to the investigation of *in planta*
46 antifungal activities of these essential oils components.
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3 **Keywords:** *Fusarium*; *Aspergillus*; *Alternaria*; *Penicillium*; mycotoxins; essential oil
4 components
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10 **Introduction**

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13 The contamination of agro-food products with mycotoxins produced by several classes of
14 phytopathogenic fungi is an age old problem which has been of particular concern over the
15 last four decades. Mycotoxins can cause both acute or chronic toxic effects and are
16 responsible for repeated episodes of food poisoning both in livestock and in humans. Of
17 worldwide relevance is the risk associated with mycotoxin contamination of cereals because
18 these plants are included in the ten major vegetable foods and the mean worldwide
19 contribution of rice, wheat and maize to the dietary energy consumption is 534, 536 and 137
20 Kcal/person/day respectively (data referred to 2003-2005, FAO Statistical Yearbook 2009). In
21 grain cereals, mycotoxins are mainly produced by fungi like *Aspergillus*, *Penicillium*,
22 *Alternaria* and *Fusarium* that colonize the plant in the field during its development and can
23 spread during the post-harvest period, producing several classes of mycotoxins as
24 secondary metabolites.
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29 Control measures for reducing mycotoxin contamination in agro-food products are therefore
30 a priority (Dayan et al. 2009; Spanjer 2008). Several pesticides widely used in plant
31 protection, such as pyrethroids, neonicotinoids, strobilurins, phenylpyrroles, avermectins,
32 milbemycins, derive from natural compounds. However, the development of new pesticides
33 is of interest to struggle with the evolution of resistant pathogens and to replace the
34 compounds lost due to the new registration requirements (Dayan et al. 2009). Plant extracts
35 and essential oils have been investigated for their antimicrobial activity (Bakkali et al. 2008;
36 Isman 2000), starting from the idea that the secondary metabolism of plants have evolved to
37 oppose pathogens (Brenner 1993). Among others, *Melaleuca alternifolia* essential oil (TTO,
38 Tea Tree Oil) has demonstrated to have inhibitory effect on phytopathogenic fungi, like
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3 *Aspergillus fumigatus*, *Fusarium solani*, *Penicillium expansum*, *Botrytis cinerea*, *Rhizopus*
4 *oryzae*, *Blumeria graminis*, *Pyrenophora graminea* (Angelini et al. 2006; Bishop and Reagan
5 1998; Bowers and Locke 2000; Inouye et al. 2000). In a previous study we have examined
6 the effects of TTO and of its major purified components (terpinen-4-ol, γ -terpinen and 1,8-
7 cineole) on two mycotoxigenic fungi, i.e. *Fusarium graminearum* and *Fusarium culmorum*
8 (Terzi et al. 2007). Because the antimicrobial activity of several essential oils has been
9 attributed to the presence of specific phenolic compounds (Arfa et al. 2006; Farag et al.
10 1989; Kim et al. 1995; Lambert et al. 2001; Moleyar and Narasimham 1992; Tsao and Zhou
11 2000), in this study the antifungal activity of five molecules that can be found in some
12 essential oils has been evaluated on twelve different strains of plant pathogenic fungi able to
13 colonize several relevant crops and to produce a wide range of mycotoxins. Moreover, as
14 essential oils and their components are known to disrupt the cellular membranes even in
15 plant cells (Kapros and McDaniel 2009), their phytotoxicity has been evaluated in a barley
16 experimental model.

31 **Materials and methods**

32 ***Fungal material***

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35 The following fungal strains were used: *Fusarium subglutinans* (ITEM 159), *Fusarium*
36 *cerealis* (ITEM 4332), *Aspergillus tubingensis* (ITEM 5014), *Fusarium verticillioides* (ITEM
37 231), *Fusarium proliferatum* (ITEM 1407), *Aspergillus carbonarius* (ITEM 4711), *Fusarium*
38 *proliferatum* (ITEM 380), *Fusarium oxysporum* (ITEM 386), *Alternaria alternata* (ITEM 762),
39 *Fusarium sporotrichioides* (ITEM 194), *Penicillium spp* (ITEM 5147), *Fusarium*
40 *sporotrichioides* (ITEM 3765), *Fusarium culmorum* (HM 169/02). This last *F. culmorum* strain
41 is a strong deoxynivalenol producer and was kindly provided by dr. Ivana Polisenska
42 (Agrotest Fyto, Ltd, Kromeriz, Czech Republic). All the remaining fungal strains were kindly
43 provided by dr. Antonio Moretti (ISPA, Institute of Sciences of Food Production, CNR-
44 National Research Council, Via Amendola, 122/O - 70126 Bari, Italy) and belong to the ISPA

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3 collection of toxigenic fungi of agro-food interest (dr. Antonio Logrieco curator). Information
4 on the origins and toxigenicity of these strains can be accessed at www.ispa.cnr.it/Collection.
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8 9 **Chemicals**

10 Eugenol ($\geq 99\%$, Mr 164.21, Fluka code 46100), thymol ($\geq 99\%$, Mr 150.22 Rieder-deHaen
11 code 16254), carvone ($\geq 98.5\%$, Mr 150.22, Fluka code 22070) terpinen-4-ol ($\geq 99\%$, Mr
12 154.25, Fluka code 86477) and 1,8-cineole ($\geq 99\%$, Mr 154.25, Fluka code 29210) were
13 purchased from Sigma-Aldrich S.r.l. (Milan, Italy)
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18 19 20 21 **Evaluation of oil component effects on *in vitro* fungal growth**

22 The mycelium growth of the fungal strains was evaluated on solid PDA medium (Liofilchem,
23 Teramo, Italy) amended with 0.5% Tween 20 and with oil component percentages ranging
24 from 0 to 1%. The experiments were conducted twice in triplicate in 60 mm Petri dishes,
25 inoculated with 8 mm PDA plugs from actively growing cultures. Mycelium growth was
26 evaluated as mean diameter measured every day from fungal inoculation up to 6 days post
27 inoculation.
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35 Analysis of variance (ANOVA) with fungal strain, the oil component, the percentage of oil
36 component as main factors were used to test the main effects and factor interactions on the
37 fungal growth. ANOVA was done using the Systat package version 9.0 (SPSS Inc., Chicago,
38 USA).
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43 The effect of different percentages of oil components was expressed as percent of inhibition,
44 the log₁₀ dose concentration–response relationships were linearized using probits and EC₅₀
45 and EC₉₀ were evaluated (Finney 1971).
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49 To evaluate the loss of viability in absence of fungal growth, eight mm PDA plugs of
50 *Fusarium cerealis* were inoculated on Petri dishes containing thymol (at concentrations of
51 0.01%, 0.023%, 0.1%) or eugenol (at concentrations of 0.014%, 0.033%, 0.1%) or carvone
52 (at concentrations of 0.017%, 0.044%, 0.1%) or 1,8 cineole (at concentrations of 0.24%,
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0.426%, 1%) or terpinen-4-ol (at concentrations of 0.07%, 0.113%, 0.1%, 0.5%), respectively. Six days post inoculation the Petri dishes were washed with 3 ml of sterile distilled water and 3 µl of water were inoculated on new PDA Petri dishes. The presence of mycelium development was checked after six days and recorded as mean diameter. The experiment was done in nine replicates and a not-treated control was included.

Evaluation of thymol effects on F. cerealis morphology.

Experimental cultures of *F. cerealis* were cultured in PDA without or with 0.1% thymol and stained with 0.01% Calcofluor White (CFW) in PBS pH 7 for 30 min. Samples were observed with an Olympus BX51 microscope with the settings of excitation at 335-380 nm and emission at > 420 nm. Images were recorded using an Olympus DP50 microscope digital camera system.

Evaluation of oil component effects on deoxynivalenol production

Thirty ml of the liquid medium described by Jiao et al (2003) amended with different percentages of oil components were inoculated with standard amounts of *F. culmorum* mycelium and left to growth for ten days in the dark at 25°C. The experiment was done in triplicate. The following treatments were considered:

Control (without any oil component addition);

Carvone at 0.025%, 0.0125%, 0.0062% concentration;

1,8-cineole at 0.25%, 0.125%, 0.062%, 0.031%;

Eugenol at 0.025%, 0.0125%, 0.0062%, 0.0031%;

Terpinen-4-ol at 0.025%, 0.0125%, 0.0062%, 0.0031%;

Thymol at 0.0125%, 0.0062%, 0.0031%

Deoxynivalenol concentration in the culture filtrate was determined by using the Ridascreen Fast DON ELISA kit (R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's

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3 instructions. For quantitative determination of fungal growth after incubation, mycelium
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5 harvested on filter paper was dried at 60°C overnight before measurement of dry weight.

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7 Analysis of variance (ANOVA) with fungal growth (mg), oil component, percentage of oil
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9 component as main factors were used to test the main effects and factor interactions on the
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11 DON production (ng g^{-1}). ANOVA was done using the Systat package version 9.0 (SPSS
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13 Inc., Chicago, USA).

14 15 16 17 ***Evaluation of inhibitory effects on plant seed germination***

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19 Twelve barley cultivars or breeding lines were used to evaluate the effects of different
20
21 percentages of oil components on seed germination. Six of the barley genotypes were
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23 hullless (Fior4508, Priora, Fior6328, Zacinto, Rattan, Fibar) and six were hulled (Fior 4503,
24
25 Aldebaran, Nure, Aiace, Tidone, Aliseo). Moreover, Fior 4503 and Fior 4508 are two barley
26
27 near-isogenic lines that only differ for the presence/absence of the seed teguments. Stocks
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29 of 100 seeds/genotype were treated for 2 hours with the following solutions:

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31 0.05% Irol Plus (Isagro Italia, S.r.l., Milan, Italy) in sterile distilled water;

32
33 0.05% Irol Plus in sterile distilled water containing 0.05% or 0.125% or 0.25% thymol or
34
35 eugenol or carvone or terpinen-4-ol;

36
37 0.05% Irol Plus in sterile distilled water containing 1% cineole.

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39 After the treatments, the seeds were placed on wetted filter paper (2 ml sterile distilled water)
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41 in a Petri dish at room temperature and the percentages of germination were evaluated after
42
43 1 week. Two replicates of the experiment were done. The data obtained were statistically
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45 analysed with Student's t-Test; the level of $P < 0.01$ was considered statistically significant.
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47 GLM (General Linear Model) analysis of germination data was performed on the data
48
49 obtained with Systat package version 9.0 (SPSS Inc., Chicago, USA).

50 51 52 53 54 55 **Results**

56 57 58 ***Evaluation of oil component effects on in vitro fungal growth***

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3 Thymol, eugenol, carvone, terpinen-4-ol and cineole showed strong fungicidal activity on
4 twelve different strains of mycotoxigenic fungi, belonging to different species and involved in
5 several plant diseases. The effects of the five oil components were individually evaluated by
6 measuring the radial growth of the fungal mycelia on solid medium amended with several 0-
7 2% concentrations of the molecules. After transformation of mycelium growth data
8 (expressed as mean percentage of growth inhibition respect to control) on a log-probit scale,
9 the EC50 and EC90 (expressed as ml of compound/100 ml medium) were calculated for
10 each oil component on each fungal strain: the results are summarized in Figure 1. Among
11 the five natural molecules tested, three groups of potency can be individuated: thymol,
12 eugenol and carvone were toxic for the fungi at the lowest concentrations (mean EC50 for
13 the three molecules = $0.017\% \pm 0.012$; mean EC90 = $0.051\% \pm 0.041$), terpinen-4-ol was
14 effective at middle concentration (mean EC50 = $0.047\% \pm 0.013$; mean EC90 =
15 $0.112\% \pm 0.043$), whereas cineole was toxic only at the highest concentration (mean EC50 =
16 $0.248\% \pm 0.167$; mean EC90 = $0.614\% \pm 0.343$). Figure 2 shows the dose-response curves for
17 the five different molecules calculated on the inhibition growth data of all the fungal strains
18 considered. All the tested molecules have equal maximal efficacy, but they are different for
19 potency, defined as the range of concentrations over which a chemical produces increasing
20 responses.

21 ANOVA made on *in vitro* growth of fungi treated with different percentages of thymol,
22 eugenol and carvone showed a significant effect for all the factors tested, i.e. the fungal
23 strain, the oil component, the oil component percentage, and for their interactions, at
24 $p < 0.001$. The percentage was the most influential factor, accounting for as much as 89.74%
25 of the total variance, followed by the oil component (1.99%), the strain (0.2%) and by their
26 interaction (strain x oil component = 0.3%; strain x percentage = 0.82%; oil component x
27 percentage = 4.76%; strain x oil component x percentage = 1.18%). ANOVA on growth data
28 of fungi treated with cineole and terpinen-4-ol showed significant effects of the percentage, of
29 the fungal strain and their interaction. For terpinen-4-ol the percentage is the most important

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3 factor, explaining 93.7% of the total sum of squares, followed by the strain-percentage
4 interaction (2.94%) and strain (1.85%). For cineole, the percentage explains 89.9% of the
5 sum of squares, the fungal strain the 2.26% and their interaction 5.39%.
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9 Further experiment was done to check the viability of the fungi in absence of mycelium
10 growth. This was evaluated in a model system constituted by a *Fusarium cerealis* strain
11 grown on PDA medium containing three different levels of each oil components
12 (corresponding to the EC50, EC90 and EC100). Table 1 reports the data obtained: after
13 treatment with 1% cineole, 0.5% terpinen-4-ol and 0.1% eugenol, thymol and carvone no
14 regrowth was observed. On the contrary, regrowth was observed from the other samples.
15 Therefore, it is possible to conclude that EC100 has fungicidal effect, whereas the other
16 treatments (EC50 and EC90) are fungistatic.
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25 26 27 ***Evaluation of oil component effects on deoxynivalenol production***

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29 The effects of fungistatic concentrations of oil components on DON yield were evaluated in
30 an experimental model constituted by *F. culmorum* inoculated in a sucrose-containing liquid
31 medium that activates *Tri* gene expression and induces tricothecene biosynthesis (Jiao et al,
32 2003). The mean DON content is 151.5 ng g⁻¹ in the control and 46.3 ng g⁻¹ in the treated
33 samples. The mean mycelium content is of 765 mg in the control and of 60 mg in the treated
34 samples. ANOVA showed that the DON production in control samples is significantly
35 different from that observed in all the treatments (R=0.896; P=0.000). On the contrary,
36 ANOVA showed that there are no significant differences in the DON production among the
37 different treatments.
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49 50 ***Evaluation of thymol effects on F. cerealis morphology***

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52 The CFW staining shows in control samples (Figure 3A) an expected pattern of chitin
53 deposition with preferential staining at conidia and visualization of intracellular septa within
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3 the hyphae, whereas in samples incubated with 0.1% thymol the chitin is dispersed in the
4
5 whole mycelium (Figure 3B).
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8 9 ***Evaluation of inhibitory effects on plant seed germination***

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11 Experiments of seed germination were performed to test the potential phytotoxic effect of the
12
13 five oil components. Germination rate was calculated on 100 seeds (two replicates) of each
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15 of the seven hulless and seven hulled barley genotypes per treatment. The oil component
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17 percentages that were used to treat the seeds before germination tests were fixed to be
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19 equal or higher than the mean LC90s found in fungal growth experiments (see Materials and
20
21 Methods). Student's t-Test applied to the data showed that the mean germination values of
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23 seeds treated with percentages of eugenol or carvone or thymol or terpinen-4-ol equal or
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25 higher than 0.25% are significantly ($p < 0.01$) different from the controls. On the contrary,
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27 percentages of these oil components equal or lower than 0.125% did not affect seed
28
29 germination. The mean germination value of barley seeds treated with 1% cineole was not
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31 significantly different from the control.
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34 GLM analysis applied to the germination data showed that the oil percentage is the main
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36 factor affecting total variance, followed by the oil component and by the presence/absence of
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38 the hull (Figure 4). On the contrary, the barley genotype did not show significant effects.
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42 43 **Discussion**

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45 Plant essential oils are a potentially useful source of antimicrobial compounds, however their
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47 composition can vary greatly depending upon several factors, like genetic variability inside
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49 plant species, the geographical region of plant cultivation, the occurrence of biotic and
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51 abiotic stresses, the phenological stage of the plant and its chemotype, the method of drying
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53 and the method of extraction of the oil (Cosentino et al. 1999; Jerkovic et al. 2001; Labra et
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55 al. 2004; Rota et al. 2008; Zheljazkov et al. 2008). Standardization and comparison of results
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57 after application of essential oils as antifungal agents is therefore difficult, due to the variable
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3 composition of essential oils in their main constituents. For this reason in our work we
4 focussed on the use of a set of five pure oil components (thymol, eugenol, carvone, terpinen-
5 4-ol and cineole), as potentially fungicide agents against mycotoxigenic plant pathogenic
6 fungi.
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10 The choice of these molecules has been suggested by two order of reasons: all these
11 molecules are widely used as medicinal antimicrobial agents and disinfectants (Voda et al.
12 2003) and additionally they are commercially available at relatively low prices.
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15 The choice of a set of mycotoxigenic fungi that are of great relevance for Mediterranean
16 crops has been suggested by the need to explore new strategies for the control and
17 reduction of mycotoxins in agro-food chains.
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20 All the tested essential oil components, depending on their concentration, showed antifungal
21 activity, measured as *in vitro* mycelium growth, on all the twelve fungal strains studied. The
22 fungistatic or fungicide effect was dose-dependent. The five natural molecules showed
23 different level of potency, according to the following scale:
24 thymol>eugenol>carvone>terpinen-4-ol>1,8-cineole. This is in agreement with Kurita and
25 Koike (1983) who found that antifungal activity of essential oils depends on their major
26 components and follows the rule:
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28

29 Phenols>alcohols>aldehyde>ketones>ethers>hydrocarbons
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32 Thymol, a monoterpene phenol occurring in the volatile oil of *Thymus vulgaris* L., had the
33 highest level of toxicity against all the fungi studied, with mean EC90 = 0.0305 ± 0.002.
34 Thymol has been found to affect mycelium morphology, with changes in the localization of
35 chitin within the hyphae. The outstanding position of thymol as antimicrobial agent has
36 already been pointed out in studies focused on environmental moulds (Segvic Klaric et al.
37 2007), bacteria (Dorman and Deans, 2000) and foodborne pathogens (Friedman et al. 2002;
38 Rasooli et al. 2004; Sokeman et al, 2004). Our study agrees with the findings that most of the
39 antimicrobial activity of essential oils from *Thymus* genus appears to be associated with
40 phenolic compounds as reported by Cosentino et al. (1999), Davidson and Naidu (2000),
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3 Rota et al. (2008), and Skocibusic et al. (2006). The other phenolic component of essential
4 oil presently/here studied is eugenol, a methoxy phenol with a free hydroxyl group that is
5 present in extracts of several plant species, like cloves, nutmeg, cinnamon, bay leaf. Eugenol
6 showed high level of toxicity against phytopathogenic fungi, with mean EC90 = 0.0395 ±
7 0.012. Strong fungicidal effects were shown even by the monoterpene carvone (mean EC90
8 = 0.0892 ± 0.054).

9
10
11 Terpinen-4-ol, the primary active ingredient of TTO, confirmed its fungicidal activity,
12 previously found against other plant pathogenic fungi (Terzi et al. 2007). Among the five
13 molecules tested, the cyclic ether 1,8-cineole, dominant component of *Eucalyptus globulus*
14 oil, is active only at the highest concentrations: this monoterpenoid completely prevents the
15 fungal growth at concentration lower than 1%.

16
17
18 In our work we have demonstrated that the DON production in *F. culmorum* treated with sub-
19 lethal concentrations of all the essential oil components is significantly reduced in
20 comparison with control. Several publications have reported that essential oil compounds
21 can cause a reduction in mycotoxin production. Soliman and Badeaa (2002) found that
22 thyme oil was effective in the prevention of toxin production in wheat inoculated with
23 toxigenic fungi during storage. Phenolic compounds have antifumonisin activity and several
24 hypotheses have been suggested to explain their mechanism of action, like an increase in
25 the lag phase prior to fungal growth (Samapundo et al. 2007) or the impact of antioxidant
26 activity on aflatoxin biosynthesis (Dambolena et al. 2008). Phenolic compounds can inhibit
27 even aflatoxin production by altering the modulators of mycotoxin biosynthesis or by altering
28 signal transduction pathway (Holmes et al. 2008). Molecular properties like lipophilicity, molar
29 refractivity and saturated area were demonstrated to be correlated with antifumonisin activity
30 (Dambolena et al. 2011).

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33 To evaluate the potential toxic effect of the five molecules against crops, the germinability
34 was tested on a panel of hulled and hullless barley seeds treated with the essential oil
35 components. From the results obtained, it can be concluded that thymol, eugenol, carvone
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3 and terpinen-4-ol at concentration of 0.125% did not significantly affect barley seed
4 germination. The same was for 1% cineole. These levels of concentrations are equal or
5 greatly lower than the fungicidal concentrations for each oil component: the use of these
6 molecules as natural fungicides is therefore compatible with the health of the treated plants.
7
8 Statistical analysis showed that there is an interaction between the presence of the oil
9 components and the seed hull. This effect of the hull was already observed in the previous
10 work of Terzi et al. (2007), confirming that hullless genotypes were less affected by
11 treatments in comparison with hulled ones.
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13
14 From the point of view of the fungal strains studied, there is variability of the different species
15 in the sensitivity to the oil components, as demonstrated by ANOVA. The greatest variability
16 can be found in response to carvone, the least in response to thymol. Interestingly, the two
17 strains of *Fusarium proliferatum* (ITEM 380 and ITEM 1407), both isolated on maize and
18 belonging to different chemotypes, showed high level of resistance to carvone, 1,8-cineole
19 and terpinen-4-ol.
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22 In conclusion, in our work we have demonstrated that thymol, eugenol and carvone are
23 highly active for the *in vitro* control of fungal species that are responsible for mycotoxin
24 contamination in crops with worldwide relevance. The fact that these compounds are
25 commercially available at low prices and that are characterized by low level of phytotoxicity
26 support the hypothesis/possibility of their use for crop protection. Therefore, these evidences
27 support further work aimed to the validation of the use of thymol, eugenol and carvone in
28 open field trials and to the evaluation of their commercial applicability as alternative to the
29 use of synthetic fungicides in organic farming.
30

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 **Acknowledgements**

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53 This work was supported by the Emilia-Romagna project “Riduzione del rischio di
54 contaminazione da micotossine nei cereali” and by a sponsorship from Milte Italia.
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29 **Legend to figures**

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31 Figure 1. The EC50 and EC90 (expressed as ml of compound/100 ml medium) of thymol,
32 eugenol, carvone, 1,8-cineole and terpinen-4-ol on *in vitro* mycelium growth of twelve
33 mycotoxigenic fungi were determined measuring the radial mycelium growth 6 days after
34 inoculation from six replicates/fungal strain. In the Figure are reported the means and the
35 standard deviations obtained from all the data.
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40 Figure 2. Relationships between the percentage of oil component and the percentage of
41 fungal growth. The dose-responses curves for the five oil components were obtained from
42 the fungal growth percentages calculated respect to the controls and linearized plotting
43 concentration on a log scale and response on a probit scale. For thymol the R^2 was 0.9446,
44 for eugenol 0.8657, for carvone 0.7216, for terpinen-4-ol 0.7661 and for cineole 0.7933.
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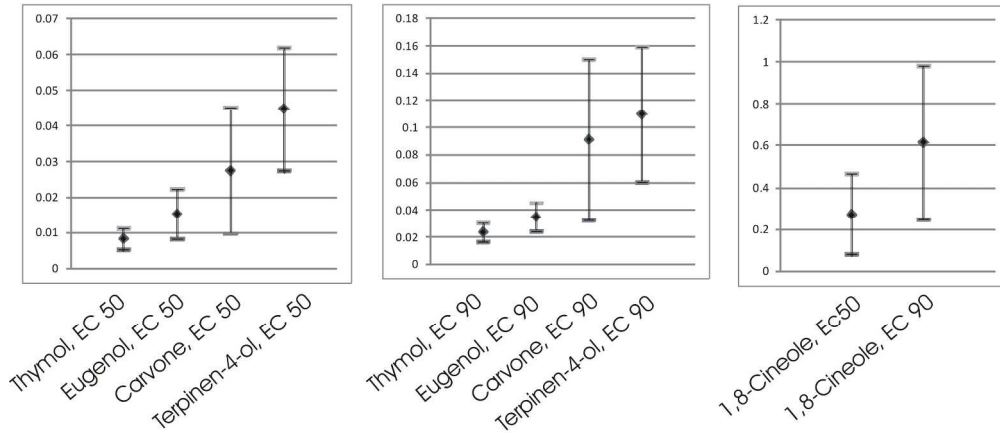
51 Figure 3. Calcofluor–White staining of control (A) and thymol-treated (B) *Fusarium culmorum*.
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3 Figure 4. Mean germination percentages of hulless (n) vs hulled (v) barley seeds treated with
4 thymol or eugenol or carvone or terpinen-4-ol or 1,8-cineole at concentration ranging from 0
5 to 1% (General Linear Model, GLM analysis).
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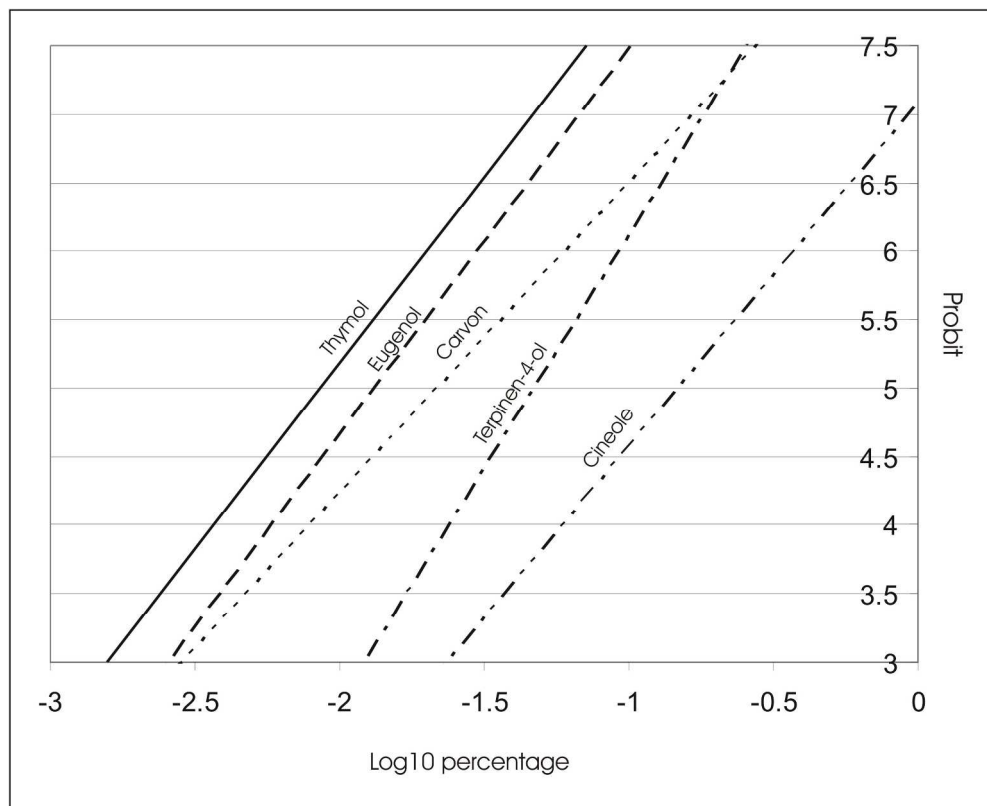
Table 1. Evaluation of fungicidal/fungistatic effects of essential oil compounds on *F. culmorum* mycelium. After the indicated treatments, the mycelium was washed, plated out and the viability of the fungus was evaluated measuring the mean radial growth for six days. The data, expressed in cm, are the means of nine replicates.

Control	1% 1-8 cineole	0,426% 1-8 cineole	0,24% 1-8 cineole
5.2±0	0±0	0.067±0.025	1.19±0.27
Control	0,5% terpinen-4-ol	0,1% terpinen-4-ol	0,071% terpinen-4-ol
5.2±0	0±0	1.17±0.26	2.18±0.53
Control	0,1% eugenol	0,033% eugenol	0,014% eugenol
5.2±0	0±0	1.54±0.11	4.54±0.29
Control	0,1% thymol	0,023% thymol	0,01% thymol
5.2±0	0±0	1.21±0.19	3.47±0.21
Control	0,1% carvone	0,044% carvone	0,017% carvone
5.2±0	0±0	0.28±0.083	4.47±0.8



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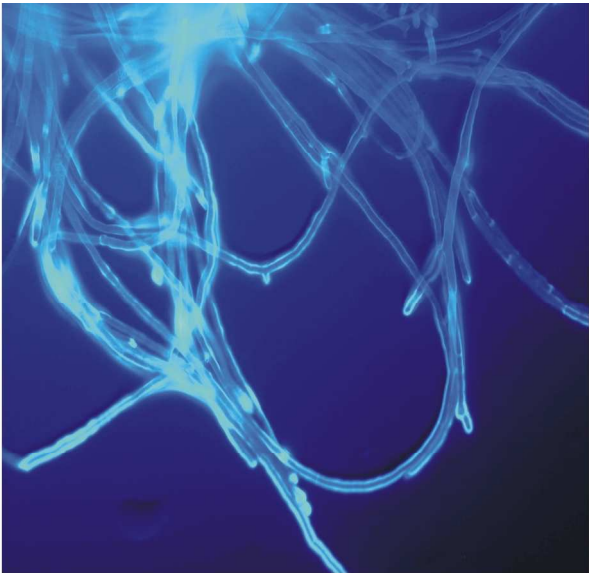


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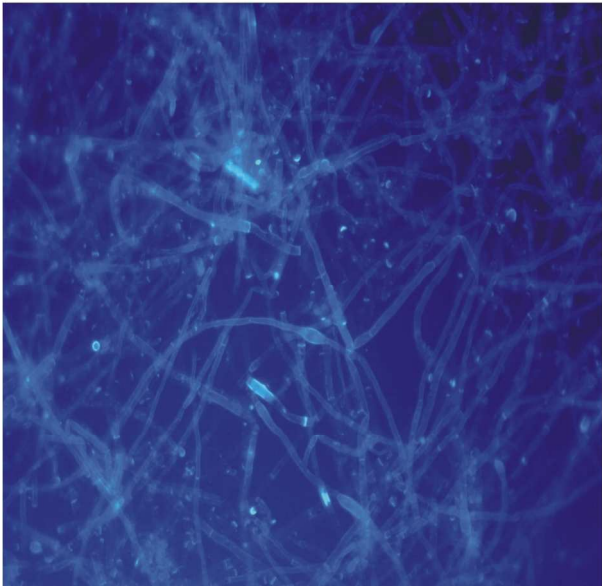
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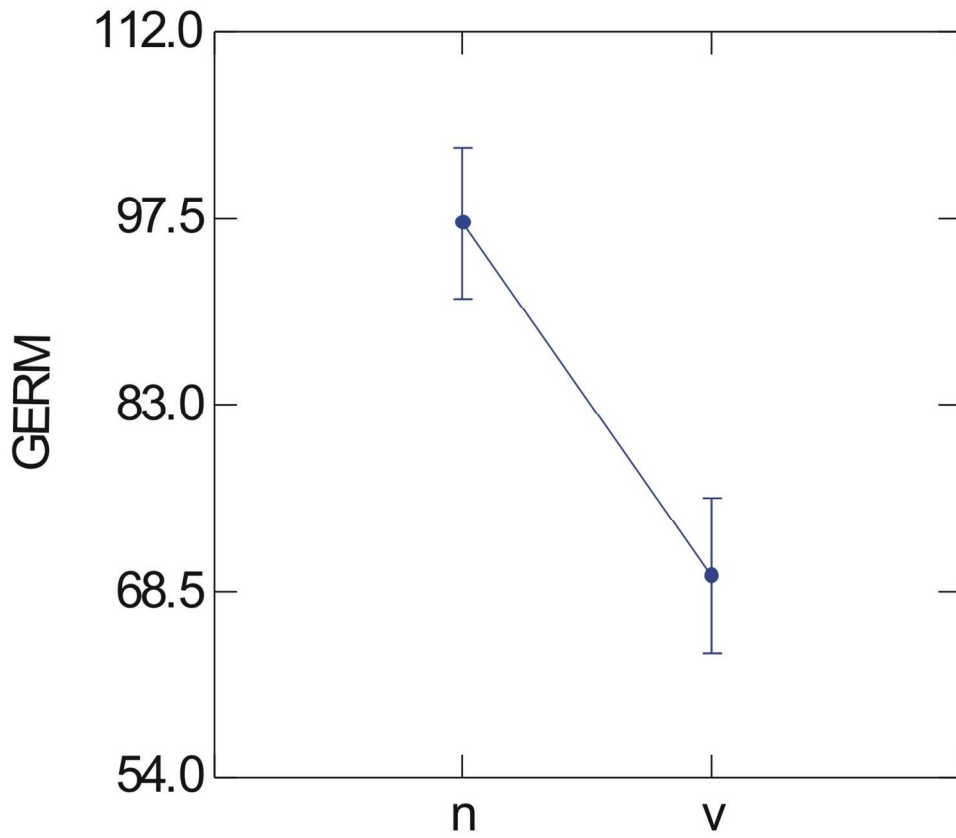
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